To investigate how the evolved architecture of the ribozyme leads to cooperative folding, we studied the equilibrium folding of wild type and mutant ribozymes at single residue resolution using hydroxyl radical footprinting. The results confirmed the collapse transition of the wild type ribozyme is specific, producing native-like interactions. Footprinting experiments on mutants lacking one or more tertiary contacts showed that perturbations to the network of tertiary interactions change the outcome of the initial collapse transition, increasing the population of non-native intermediates. For instance, weakening the central triple helix changed the assembly pattern of the three helical domains and impaired docking of the substrate binding domain with the rest of the structure. Perturbation of a peripheral interaction, on the other hand, caused partial misfolding of the active site after the initial collapse.
Our results suggest that natural selection of architectures conducive to cooperativity in the collapse transition may be a general mechanism to ensure folding specificity in evolved RNAs.

3277-Pos Board B138
Tracking Bacterial Riboregulation by DsrA Noncoding RNA
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Small regulatory noncoding RNAs (sRNA) are evolutionarily conserved molecules that act by base pairing with mRNAs in response to environmental stress. They can affect cell physiology in numerous ways, including changes in cell morphology, remodeling the bacterial cell surface and decreasing growth rates. These stress-related sRNAs require the RNA chaperone protein Hfq for their regulatory function. Most of the sRNAs, for which a mechanistic understanding is available, act as inhibitors of translation by base pairing with their mRNA target around the ribosome-binding site (*rbs*). Nevertheless, there are also some examples, as in the case of DsrA, where the sRNA plays a role of both a translational inhibitor of the bacterial actin-like MreB, and a translational activator by exposing the mRNA *rbs* and promoting ribosome binding to *rpoS* mRNA (encoding the sigma S transcriptional factor). We recently observed that the *E. coli* DsrA sRNA is present in few copies in the bacterial cell. For this reason, the ability of the sRNA to bind an mRNA and regulate protein translation can be viewed essentially as a single-molecule process in individual cells, probably contributing to variation from cell to cell in a population. Our aim is to analyze the dynamics of DsrA-dependent translation in response to various stresses at the single molecule level using single-molecule FRET assays. Dye-labeled synthetic RNA fragments of DsrA and of its targets were used to follow and quantify Hfq-dependent RNA annealing. The speed and efficiency of annealing are directly influenced by physico-chemical parameters, such as temperature, and changes in sRNA concentration encountered during physiological stresses.

3278-Pos Board B139
RNA Pseudoknots Obtained by Means of Knowledge-Based Interaction Potentials
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In order to take into account the angular dependence of hydrogen bonding, we modified some of the effective pair potentials (EPP) that were previously extracted from a series of crystallographic structures of large RNA molecules. The new set of EPP were used as the main constituents of a Monte Carlo simulation model. Our simulated chains are consistent with the experimental structures obtained by NMR.

3279-Pos Board B140
Frameshifting Efficiency is not Determined by the Mechanical Stability of RNA Pseudoknots
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Ribosomes translate messenger RNA
(mRNA) in 3-nucleotide steps, maintaining an open reading frame until a stop codon is reached. Programmed −1 translational frameshifting, whereby the ribosome is forced backward by 1 nt to shift the reading frame, occurs in many viruses. The resulting expression of two proteins from a single mRNA at a fixed ratio is essential for viral propagation. Frameshifting depends on two mRNA structures: a slippery sequence and a downstream pseudoknot. Recent work suggests frameshifting pseudoknots provide greater barriers to unfolding by the ribosomal helicase (1), likely a function of their increased mechanical stability. The mechanical stability of a panel of 8 pseudoknots, associated with different frameshifting efficiencies, was investigated by repeatedly unfolding and refolding single pseudoknot molecules held under tension by optical tweezers. Pseudoknot unfolding was characterized by measuring the associated molecular contour length changes, unfolding forces, and force-dependent kinetics. Most of the pseudoknots we examined unfold at high force (~30-50 pN). The corresponding contour length changes were consistent with native pseudoknot unfolding. Most often the unfolding occurred in a single step, but for some pseudoknots the dominant unfolding pathway occurred via a partially-folded intermediate at lower force (~15-20 pN). Surprisingly, we found no obvious correlation between frameshifting efficiency and pseudoknot mechanical stability, indicating that some other property must determine the frameshifting efficiency. These results extend our understanding of the mechanics of RNA pseudoknot structure formation, and the relation between pseudoknot folding and programmed translational frameshifting.
(1) Namy, O., Moran, S.J., Stuart, D.I., Gilbert, R.J. & Brierley, I. A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. *Nature* **441**, 244-247 (2006).

3280-Pos Board B141
Kinetic Mechanism for the Conformational Switch Between Bistable RNA Hairpins
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Transitions between different conformational states may be intrinsic to RNA catalytic and regulatory functions. In this study, we use Kinetic Monte Carlo method to investigate the refolding mechanism for the conformational switch between bistable RNA hairpins. By analyzing the first passage time of each conformation, we find that there exist three refolding pathways for the hairpin: the unfolding-folding, the tunneling and the pseudoknot-assisted pathways. Depending on the location of the rate-limiting base stack and the relationship between the two bistable hairpins. the competition between the three types of pathways can lead to a single dominant pathway or multiple dominant pathways. The activation energy extracted from the rate-temperature Arrhenius plot support our refolding pathway analysis and the results are further validated through comparisons with experimental data.

3281-Pos Board B142
Solution Structures of Flexible RNA Molecules in Mono- and Divalent Salt
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Cells transmit and process genetic information using single-stranded RNA (ssRNA); these molecules realize their function through dynamic structural changes such as folding, association with binding partners, and conformational switching. Disordered, un-basepaired states of RNA play a key role in these dynamic structural transitions, yet most relevant measurements of single-stranded oligonucleotides have focused on DNA. Thus, we measure and compare the conformations of chemically similar ssRNA and ssDNA oligonucleotides lacking secondary structure. SAXS curves and smFRET efficiencies of rU40 and dT40 in 100mM NaCl are well-described by a simple wormlike chain model. We detect subtle but significant differences between the contour and persistence lengths of rU40 and dT40 that agree with predictions based on relative sugar pucker preferences of the two nucleotides. To compare the polyelectrolyte properties of ssRNA and ssDNA, we report persistence lengths derived from smFRET data acquired over a wide range of ionic conditions. We find that the apparent charge screening efficiency of divalent magnesium is anomalously large compared to monovalent sodium in both ssRNA and ssDNA. This strong interaction between divalent ions and disordered RNA presents an important problem for polyelectrolyte theory and challenges our understanding of how ions influence RNA folding.

3282-Pos Board B143
Glass-Like Behavior of Magnesium Ions Inside RNA APTamers
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RNA is known to be strongly dependent on Magnesium for stability. In this computational study, we explore the effects of Magnesium concentration on the SAM I riboswitch with several microsecond-plus simulations at varying concentrations. Individual Magnesium binding events are shown to influence the RNA conformation through tertiary bridging interactions. We characterize the behavior of Magnesium near RNA for use in structure-based models, and note that it is glassy.